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A retrospective analysis of the added value of the rat two-generation reproductive toxicity study versus the rat subchronic toxicity study

Gemma Janer*, Betty C. Hakkert, Aldert H. Piersma, Theo Vermeire, Wout Slob

National Institute of Public Health and the Environment (RIVM), 3720 BA Bilthoven, The Netherlands

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Abstract

This study aims to evaluate the added value of the two-generation reproductive toxicity study when a subchronic study (90-day repeated dose toxicity study) is available. The analysis includes a total of 47 reproductive toxic and 75 non-reproductive toxic substances, for which a two-generation study was available. For each of these compounds the outcomes of both study types were compared, in view of the question what the impact would have been both for the derived NOAEL and for classification regarding toxicity to fertility. On average, only a small difference (less than twofold) in overall NOAELs was found between the rat two-generation study and the rat subchronic study. For individual compounds the differences could be larger (up to around a factor of 10), but differences of this magnitude equally occur between NOAELs of subchronic studies (testing the same substance). The two generation study did have an impact on classification for toxicity to fertility: about one-third of the substances shown to be toxic to fertility in the two-generation study did not show any sign of that in the 90-day study. If the subchronic study did show toxicity to reproductive organs this often occurred at (much) higher doses than other toxic effects in the same study. Therefore, apart from including more fertility endpoints, a larger dose spacing (or more dose groups) in the subchronic study might increase its detection rate of fertility toxic substances. The consequences that these findings may have for risk assessment and risk management are discussed, especially in the context of REACH.

Keywords: Two-generation study; Subchronic study; Reproductive toxicity; Developmental toxicity; NOAEL; Classification and labeling; Risk assessment

1. Introduction

Reproductive toxicity testing according to most current international guidelines involves a prenatal developmental toxicity study in a rodent and a non-rodent species, as well as a one- or two-generation reproduction toxicity study. The latter studies are very cost- and time-intensive and require relatively large numbers of animals.

The European Union has passed into law a new regulation for industrial chemicals produced in excess of 1 tonne per annum: the REACH legislation (registration, evaluation and authorization and restriction of chemicals) that will enter into force on June 2007 (http://eur-lex.europa.eu/LexUriServ/site/en/oj/2006/l_396/l_39620061230en00010849.pdf). REACH has the ambitious expectation to fill the knowledge gaps for more than 30,000 existing substances. Testing requirements depend on the production volume for each substance (see Appendix A) [1]. On the basis of a draft version of REACH, it has been estimated that the two-generation study will be required for 7.5% of all substances to be evaluated and will use 37.5% of the laboratory animals under REACH. Reproductive and developmental toxicity testing together may even require around 70% of all experimental animals used under REACH [2]. Concern has already been expressed on the difficulties to carry out a complete reproductive toxicity test program for all these chemicals [3], and from this point of view the development of simplified reproductive toxicity testing appears highly desirable.

Two different endpoints, fertility and developmental toxicity, can lead to the classification for reproductive toxicity as established by the European Dangerous Substances Directive 67/548/EEC, although this study will focus mainly on toxicity to fertility. When a (potential) hazard for fertility is identified, substances are classified in one of the three categories for reproductive toxicity according to the Directive 67/548/EEC (see

* Corresponding author. Tel.: +31 30 2743020; fax: +31 30 2743242.
E-mail address: Gemma.Janer@RIVM.nl (G. Janer).

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Appendix B for details). Substances classified into Categories 1 and 2 are assigned an R60 label ("may impair fertility"), and substances classified into Category 3 are assigned an R62 label ("possible risk of impaired fertility"). These labels can lead to restrictions on the use of a substance or mixtures containing it. Within REACH, classification and labeling serves as a trigger for performing a risk assessment and in some cases (e.g. Categories 1 or 2 reproductive toxicants) for establishing an authorization process.

In a risk assessment the different animal studies available provide the basis to obtain an overall NOAEL (no-observed-adverse-effect-level) for that substance. This NOAEL forms the basis for deriving a DNEL (derived-no-effect-level, which is the term used in REACH), by applying uncertainty factors, e.g. for inter- and intraspecies differences.

After more than 20 years of chemical testing, considerable amounts of toxicological data have been generated. In view of the upcoming changes in regulation, it may be helpful to use the existing toxicological data to evaluate the efficiency of the testing strategy so far. A recent report by the TERA group (toxicology excellence for risk assessment [4]) compared the NOAELs obtained in a rat chronic and a rat two-generation study for a series of chemicals. The analysis showed that, in general, lower NOAELs were obtained in rat chronic than in rat reproductive toxicity studies [4]. However, most of the chemicals included in the database were not toxic to reproduction (i.e., only 9 out of 128 substances were classified as toxic to reproduction by 67/548/EEC Appendix A or U.S. California E.P.A.). In the present study, existing data will be retrospectively evaluated to assess the added value of the two-generation reproductive toxicity study over the subchronic study (90-day repeated dose toxicity study). In contrast with the TERA report, special emphasis will be put on substances classified as toxic to reproduction. Furthermore, not only the impact on the derived NOAEL, but also on the classification for toxicity to fertility will be assessed.

2. Methods

2.1. Data collection

2.1.1. Reproductive toxicants

We constructed a database with rat subchronic (90 days) and rat reproductive (two-generation) studies for substances classified as toxic to fertility (R60 or R62) or development (R61: "may cause harm to unborn child", or R63: "possible risk of harm to unborn child") up to and including the 30th Adaptation to Technical Progress of Appendix A of the EU Directive 67/548/EEC, as well as substances that are known to cause male and/or female reproductive toxicity according to the US State of California Environmental Protection Agency [5]. Publicly available, peer-reviewed national or international documentations were preferably used (Table 1). When no or insufficient data for a specific substance were found in these sources, we searched in the IUCLID files (International Uniform Chemical Information Database; http://ecb.jrc.it/esis/), the open literature (Medline), in the publicly available European Chemicals Bureau (ECB) meeting reports and working documents (http://ecb.jrc.it), and in the confidential evaluation files used for classification and labeling within the Dutch National Institute of Public Health and the Environment (RIVM). In order to keep the confidentiality of the data, no identification of the latter substances will be reported.

The EU Directive 67/548/EEC Appendix A contains 181 substances classified for reproductive toxicity (effects on fertility or development). Some of these substances are very closely related (e.g. cadmium chloride and cadmium fluoride). Therefore, we reduced the list in Appendix A to 140 substances by grouping compounds with these two criteria: they were different salts of the same chemical, or they were active metabolite and parent compound. Despite the number of sources screened for the analysis, the required toxicity data (reproductive toxicity studies or, at least, hazard assessments) could not be found for 11 substances out of the 140 substances included in Appendix A. These data are likely to be held by EU members other than The Netherlands in confidential files for classification and labeling and for new substances notifications.

Among the substances for which we found toxicological data, approximately one-third had been tested in a two-generation rat study (42 out of 140). The EU Technical Commission on Classification and Labeling had classified most of the remaining substances as toxic to fertility based on the effects observed in other rat studies (e.g. one-generation, subacute, and (sub)chronic studies) or based on studies in other species. The classification as toxic to development was, for most of the substances, based on developmental toxicity studies. Finally, in a few cases, classification was based on structural similarities with other classified substances.

The list of substances known to cause reproductive toxicity by California EPA included 56 substances that were not present in the EU 67/548/EEC Directive Appendix A. Thirty-five of these substances are drugs (medicinal or abuse). A two-generation study is rarely performed for such substances, and available data is likely to be confidential. Therefore, we did not include drugs in our database. Out of the remaining substances, a two-generation study was found for five substances. In the remaining 16, other studies had shown reproductive toxicity.

Altogether a two-generation study was found for 47 substances considered as reproductive toxicants.

2.1.2. Non-reproductive toxicants

A comparable database was created for a subset of substances for which a two-generation study is available, but which were not classified for either toxicity

Table 1
Peer-reviewed national or international data sources used

Source	Webpage
EU Risk Assessment Reports for Existing Substances (RAR)	http://ecb.jrc.it/esis/
OECD Screening Information Dataset for High Production Volume Substances (SIDS)	http://www.chem.unep.ch/irptc/sids/OECDSIDS/sidspub.html
Joint Meeting on Pesticides Residues (JMPR)	http://www.inchem.org/pages/jmpr.html
Environmental Health Criteria Monographs (EHC)	http://www.inchem.org/pages/ehc.html
Concise International Chemical Assessments Documents (CICAD)	http://www.inchem.org/pages/cicads.html
US Agency for Toxic Substances and Disease Registry (ATSDR)	http://www.atsdr.cdc.gov/toxpro2.html#bookmark05
US National Toxicology Program—Center for the Evaluation of Risks to Human Reproduction (NTP)	http://cerhr.niehs.nih.gov/reports/index.html
Canadian Pest Management Regulatory Agency (CPMRA)	http://www.pmra-arla.gc.ca/english/pubs/reg-e.html
Health Council of the Netherlands (HCN)	http://www.gr.nl/
German Advisory Committee on Existing Chemicals of Environmental Relevance (BUA)	http://www.hirzel.de/bua-report/download_53_99.html
California EPA Evaluation Reports (California EPA)	http://www.oehha.org/prop65/hazard_ident/hazard_id.html